

Tumoral Ki67 and PSMA Expression in Fresh Pre-PSMA-RLT Biopsies and Its Relation With PSMA-PET Imaging and Outcomes of PSMA-RLT in Patients With mCRPC

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Abstract

In this retrospective observational study we focused on the failure of prostate specific membrane antigen-radioligand therapy. Therefore we evaluated the correlation between PSMA uptake on positron emission tomography/computed tomography and PSMA protein expression on IHC on fresh biopsy lesion of mCRPC-patients. Secondly we PSMA uptake on PET/CT, protein expression of PSMA and Ki67 were associated with the therapeutic outcome of PSMA-RLT.

Introduction: Prostate specific membrane antigen (PSMA) directed radioligand therapy (RLT) is a novel therapy for metastatic castration-resistant prostate cancer (mCRPC) patients. However, it is still poorly understood why approximately 40% of the patients does not respond to PSMA-RLT. The aims of this study were to evaluate the pretreatment PSMA expression on immunohistochemistry (IHC) and PSMA uptake on PET/CT imaging in mCRPC patients who underwent PSMA-RLT. We correlated these parameters and a cell proliferation marker (Ki67) to the therapeutic efficacy of PSMA-RLT. **Patients and Methods:** In this retrospective study, mCRPC patients who underwent PSMA-RLT were analyzed. Patients biopsies were scored for immunohistochemical Ki67 expression, PSMA staining intensity and percentage of cells with PSMA expression. Moreover, the PSMA tracer uptake of the tumor lesion(s) and healthy organs on PET/CT imaging was assessed. The primary outcome was to evaluate the association between histological PSMA protein expression of tumor in pre-PSMA-RLT biopsies and the PSMA uptake on PSMA PET/CT imaging of the biopsied lesion. Secondary outcomes were to assess the relationship between PSMA expression and Ki67 on IHC and the progression free survival (PFS) and overall survival (OS) following PSMA-RLT. **Results:** In total, 22 mCRPC patients were included in this study. Nineteen (86%) patients showed a high and homogenous PSMA expression of >80% on IHC. Three (14%) patients had low PSMA expression on IHC. Although there was limited PSMA uptake on PET/CT imaging, these 3 patients had lower PSMA uptake on PET/CT imaging compared to the patients with high PSMA expression on IHC. Yet, no correlation was found between PSMA uptake on PET/CT imaging and PSMA expression on IHC (SUVmax: $R^2 = 0.046$ and SUVavg: $R^2 = 0.036$). The 3 patients had a shorter PFS compared to the patients with high PSMA expression on IHC (HR: 4.76, 95% CI: 1.14-19.99; $P = .033$). Patients with low Ki67 expression had a longer PFS and OS compared to patients with a high Ki67 expression (HR: 0.40, 95% CI: 0.15-1.06; $P = .013$). **Conclusion:** The PSMA uptake on PSMA-PET/CT generally followed the PSMA expression on IHC. However, heterogeneity may be

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missed on PSMA-PET/CT. Immunohistochemical PSMA and Ki67 expression in fresh tumor biopsies, may contribute to predict treatment efficacy of PSMA-RLT in mCRPC patients. This needs to be further explored in prospective cohorts.

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Introduction

Prostate cancer (PCa) is the second most diagnosed cancer and fifth leading cause of cancer deaths in men worldwide.¹ Particularly in patients with metastatic castration-resistant prostate cancer (mCRPC) the prognosis is poor.² In the last decade, several new therapies for mCRPC became available, such as enzalutamide, abiraterone, docetaxel, cabazitaxel, radium-223 (²²³Ra), and poly (ADP-ribose) polymerase inhibitors (PARPi).³ More recently, radioligand therapy (RLT) with Lutetium-177-prostate-specific membrane antigen (¹⁷⁷Lu-PSMA) received regulatory approval following the pivotal VISION trial reporting positive outcomes in mCRPC patients who were previously treated with androgen-receptor pathway inhibitors and taxane-based chemotherapy.

In recent years, prospective as well retrospective studies have shown that approximately 60% of patients benefit from PSMA-RLT.^{4–8} Unfortunately, not all patients respond to this novel therapy, despite having sufficient PSMA uptake on pretherapeutic positron emission tomography / computed tomography (PET/CT) imaging. Even PSMA ligands labeled with alpha-emitters (eg, ²²⁵Ac-PSMA-617), which have a much higher linear energy transmission, result in heterogeneous responses between patients.^{9–11} To date, several factors which can help anticipating treatment outcomes for the PSMA-RLT response have been evaluated.¹² These studies showed that the presence of visceral metastases, previous second line chemotherapy, the Eastern Cooperative Oncology Group performance status, low tumor PSMA uptake, Fluorodeoxyglucose (FDG) uptake, elevated levels of alkaline phosphatase (ALP) and the lactate dehydrogenase level (LDH) at baseline can predict treatment outcomes such as the progression free survival (PFS) and overall survival (OS).^{13–23} Yet, it is postulated that more predictive values could be extracted from the molecular characteristics of the tumor.

It was recently reported that the PSMA tracer uptake on PET/CT corresponds to the PSMA expression on immunohistochemistry (IHC) from fresh tumor biopsies in primary prostate cancer patients.²⁴ While it is assumed that a similar trend exists in mCRPC, this is still unreported to date, as tumor biopsies in advanced disease stages are not routinely performed and the overall use of cell-free DNA for precision medicine is increasing. In mCRPC patients, a high and homogenous tumor PSMA expression is particularly relevant as these patients are eligible for PSMA-RLT following PSMA PET/CT.²⁵ Consequently, tumorous PSMA expression levels may influence treatment efficacy, particularly when using targeted alpha-particle emitting radionuclides for PSMA-RLT. This is due to the short range and high linear energy transfer of alpha-emitters. Unfortunately, microscopic heterogeneity in PSMA expression of individual tumor cells is difficult to assess with PET/CT and SPECT imaging due to the limited resolution. To date, there is minimal

data available on the relationship between PSMA expression on IHC and the outcomes following PSMA-RLT.²⁶ Moreover, it is also commonly known that patients with a fast proliferating tumors have a worse prognosis.²⁷ This has also been reported for patients with neuro-endocrine tumors receiving ¹⁷⁷Lu-Octreotide RLT. In these patients, high cellular Ki67 expression as a marker of cellular proliferation, was associated with reduced OS and PFS following ¹⁷⁷Lu-Octreotide RLT. However, in patients with advanced prostate cancer, this has not been evaluated as prognostic biomarker for PSMA-RLT.

Therefore, we performed a study of mCRPC patients that had fresh pretherapeutic tumor biopsies and who underwent PSMA-RLT. We first evaluated in this study the PSMA uptake on PSMA-PET/CT of the biopsied lesion, and if this correlated with PSMA protein expression on IHC. Second, we assessed whether PSMA uptake on PET/CT, protein expression of PSMA and Ki67 were associated with the therapeutic outcome of PSMA-RLT.

Materials and Methods

Study Design and Patient Selection

This retrospective study evaluated mCRPC patients who underwent PSMA-RLT between February 2016 and September 2019 in a tertiary medical center. To be included in this study cohort, patients had received RLT with PSMA-I&T or PSMA-617 labeled with ¹⁷⁷Lu and/or ²²⁵Ac. A PSMA PET/CT and fresh tissue biopsy of a PCa metastasis acquired prior to PSMA-RLT was mandatory for inclusion. Furthermore, to have a legitimate treatment evaluation, a minimum of 6 weeks of follow up was required.

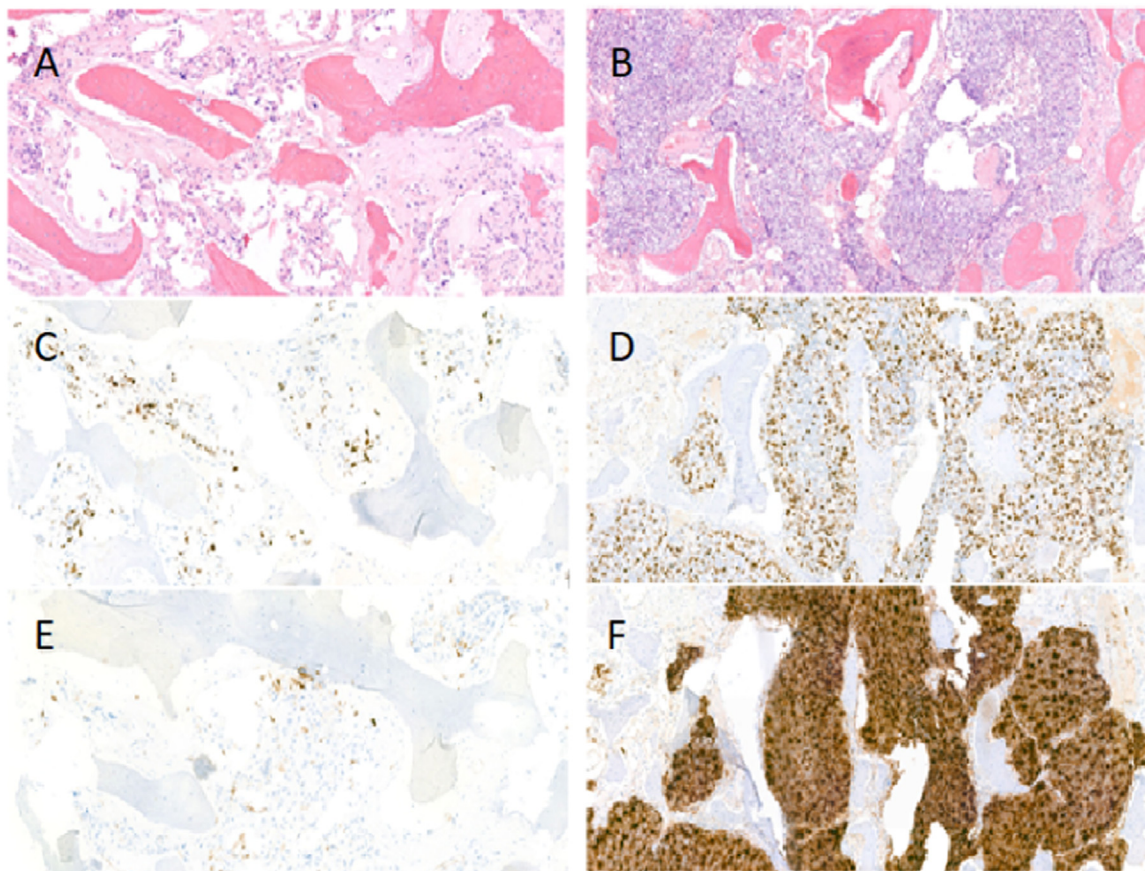
Study Outcomes

The primary outcome of this study was to evaluate the association of PSMA protein expression of tumor in pretherapeutic biopsies and the PSMA avidity of the biopsied lesion on PSMA PET/CT imaging. Secondary outcomes were to assess the relationship between PSMA and Ki67 expression on IHC and the PFS and OS following PSMA-RLT. PFS was defined as the time interval between first PSMA-RLT injection and the earliest evidence of disease progression (eg, pain increment, radiographic progression or starting a new systemic therapy).²⁸ OS was defined as time from first PSMA-RLT injection until death from any cause or last recorded date of follow-up.

Data Collection and Ethical Considerations

Demographic data, diagnostic parameters, (prior) treatments, follow up, PSMA PET/CT data and IHC data were collected and recorded in an electronic case report. The study was approved by the Medical Review Ethics Committee Region Arnhem-Nijmegen, The Netherlands. All study subjects had provided written informed

Figure 1 Examples of IHC staining in 2 patients (patient 1 = Figure A/C/E and patient 2= Figure B/D/F). Both biopsies are bone tissue and show an evident tumor location on HE-staining (A and B). C and D show a 30% versus 100% Ki67-expression in patient 1 and 2, respectively. E and F the PSMA expression is showed, 1% versus 100% in patient 1 and 2, respectively. Both patients show a high PSMA staining intensity. HE = hematoxylin and eosin; IHC = immunohistochemistry; PSMA = prostate specific membrane antigen.



consent for the Urology Biobank of the Radboudumc (CWOM 9803-0060).

Immunohistochemistry

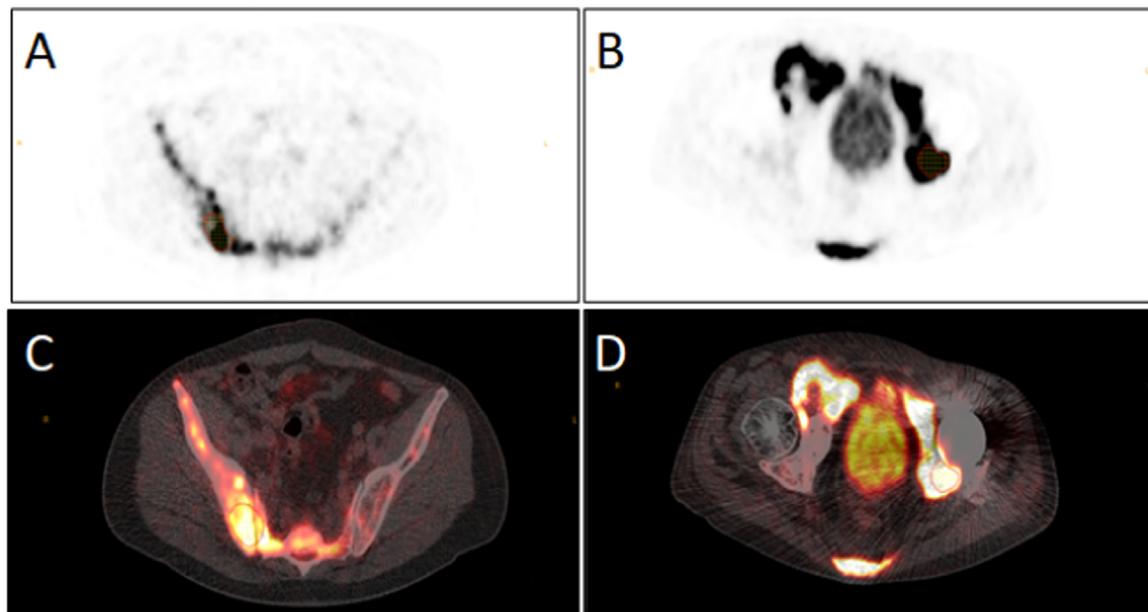
The biopsy slides were stained with hematoxylin and eosin (HE) for tumor assessment and Ki67 (dilution 1:25, DAKO, Santa Clara, CA) for cell proliferation. PSMA staining was performed using a monoclonal PSMA antibody (clone EP192, dilution 1:100, Abcam, Cambridge, UK). PSMA and Ki67 expression were scored as a percentage of immuno-positive tumor cells. The quantification was done by an experienced uropathologist (>10 years of experience). The slides were microscopically quantified based on PSMA staining intensity and PSMA expression. Staining intensity was scored on a 4-point scale (absent/weak/moderate/strong). Ki67 expression was categorized in to 2 groups; $\leq 20\%$ as low and $>20\%$ as high expression. Examples of IHC are shown in Figure 1.

PSMA-PET/CT

The PSMA uptake was assessed on the PSMA PET/CT which was carried out prior to PSMA-RLT and closest to the date of the biopsy. Patients either received a PET/CT with Gallium-68-PSMA-11 (^{68}Ga -PSMA) or Fluor-18-PSMA-1007 (^{18}F -PSMA). The acquired doses of the radiopharmakon and acquisition time were collected. The scans were analyzed with Oasis V1.0.4.11 sp3. PSMA uptake of up to 5 metastatic lesions of each organ (lymph node, bone, and visceral) were assessed. Additionally, the corresponding lesion with the biopsy location was reviewed. All lesions required a size $\geq 1.5\text{cm}$ to be considered as representative metastases.²⁹

A volume of interest (VOI) was created using a threshold of 30% of the standardized uptake value maximum (SUVmax). Subsequently, the SUVmax, SUVaverage (SUVavg), and size of the VOI were collected. Examples of PSMA PET/CT lesions are shown in Figure 2. A ratio was calculated using the PSMA uptake (overall

Figure 2 The images above correlate with the biopsy location used for the IHC staining examples in Figure 1. A and B show the PSMA PET/CT images and C and D the corresponding fused PSMA PET/CT images. VOI are shown within the red circles. Both patients showed an evident PSMA uptake on the PSMA PET/CT. The patient with low PSMA expression on IHC had a SUVmax of 18.73 and SUVavg of 8.54 (A and C). The patients with high PSMA expression had a SUVmax 27.20 and SUVavg 11.83 (B and D). IHC = immunohistochemistry; PSMA = prostate specific membrane antigen; SUVmax = standardized uptake value maximum; SUVavg = standardized uptake value average; VOI = volume of interest.



mean SUVavg) to healthy organ ratio. Patients were stratified into 2 groups based on their ratio; ≤ 3 was considered as a low and >3 was considered as a high PSMA uptake. The SUVavg of the liver or spleen were measured using a 1.5 cm region of interest for ^{68}Ga -PSMA or ^{18}F -PSMA, respectively.²⁹

PSMA-RLT

All patients were monitored at the outpatient clinic before and after each PSMA-RLT injection and received regular blood investigations including hematology, chemistry and PSA. Treatment continuation was discussed during weekly tumor board meetings, attended by medical oncologists, urologists, and nuclear medicine physicians.

Statistical Analysis

Descriptive statistical methods were used to characterize the patient cohort. For stratified data the chi-square or Fisher exact test were used. The Mann-Whitney *U* test was applied for continuous data. Correlation was measured using Pearson test. Cox Regression analysis with Hazard ratios were used to calculate the PFS and OS. A *P*-value $< .05$ was considered statistically significant. Statistical analyses were performed using SPSS software (v25) and Graphpad Prism (v5.03).

Results

Patient Characteristics

Twenty-two heavily pretreated mCRPC patients were eligible for inclusion in this study. The baseline demographics, previous therapies and PSMA-RLT details are shown in Table 1. The median time between PSMA PET/CT and tumor biopsy was 32 days (IQR: 20–84). The median time between the biopsy and the start of PSMA-RLT was 3 months (IQR: 0–5). Of the 22 patients in this study, 11 (50%) patients received ^{177}Lu -PSMA RLT, 4 (18%) patients received ^{225}Ac -PSMA RLT and 7 (32%) patients received a combination of both therapies. Patients received a median amount of 3 cycles of RLT and the total median administered activity of ^{177}Lu was 18.40 GBq and 17.50 MBq for ^{225}Ac .

PSMA Uptake on PSMA PET/CT

Nineteen (86%) patients underwent a ^{68}Ga -PSMA PET/CT scan whereas 3 (14%) patients received a ^{18}F -PSMA PET/CT scan. A total of 8 (36%) patients showed bone, lymph node, and visceral metastases. One (5%) patient showed only lymph node metastases and 6 (27%) patients showed solely bone metastases. Another 6 (27%) patients had lymph node and bone metastases and 1 (5%) patient had lymph node and visceral metastases. The overall SUV uptake of the lesions including all bone, lymph node, and

Table 1 Patients, (Previous-) Therapies and PSMA-RLT Characteristics

Patient Characteristics	Total Group (n = 22)
Age at diagnosis, years, median (IQR)	61.50 (56-68)
Gleason score at diagnosis, n (%)	
Gleason 3 + 3 (ISUP 1)	2 (9%)
Gleason 3 + 4 (ISUP 2)	4 (18%)
Gleason 4 + 3 (ISUP 3)	1 (5%)
Gleason 8 (ISUP 4)	5 (23%)
Gleason 9/10 (ISUP 5)	10 (45%)
Previous CRPC therapies	
Androgen-deprivation therapy, n (%)	22 (100%)
Taxane-based chemotherapy, n (%)	19 (86%)
Docetaxel, n (%)	10 (45%)
Cabazitaxel, n (%)	9 (41%)
Abiraterone, n (%)	15 (68%)
Enzalutamide, n (%)	20 (91%)
Radium-223, n (%)	9 (41%)
PARPi (olaparib, talazoparib), n (%)	4 (18%)
Immunotherapy (pembrolizumab, nivolumab, ipilimumab), n (%)	4 (18%)
PSMA PET/CT characteristics	
Amount of applied ⁶⁸ Ga-PSMA, MBq, median (IQR)	161 (136-174)
Amount of applied ¹⁸ F-PSMA, MBq, median (IQR)	254 (225-271)
Acquisition time ⁶⁸ Ga-PSMA, minutes, median(IQR)	56 (45-61)
Acquisition time ¹⁸ F-PSMA, minutes, median(IQR)	109 (108-116)
PSMA-RLT characteristics	
Time between CRPC and RLT initiation, months, median (IQR)	35 (22-54)
Amount of RLT cycles, median (IQR)	3 (2-4)
Only ¹⁷⁷ Lu-PSMA, n (%)	11 (50%)
Only ²²⁵ Ac-PSMA, n(%)	4 (18%)
Combination ¹⁷⁷ Lu-PSMA and ²²⁵ Ac-PSMA, n(%)	7 (32%)
Administered activity ¹⁷⁷ Lu, GBq, median (IQR)	18 (13-24)
Administered activity ²²⁵ Ac, MBq, median (IQR)	17 (6-23)

Ac = actinium; CRPC = castration resistant prostate cancer; GBq = gigabecquerel; IQR = interquartile range; MBq = megabecquerel; ISUP = International Society of Urological Pathology; PARPi = poly (ADP-ribose) polymerase inhibitors; PSMA = prostate specific membrane antigen; RLT = radioligand therapy; Lu = lutetium.

visceral metastases on the PSMA PET/CT was high. A median overall SUVmax of 39.59 (IQR: 23.17-47.07) and a median overall SUVavg 20.00 (IQR: 11.96-25.85) was found. Table 2 provides an overview of the separate SUV values for lymph node, bone, and visceral metastases.

The median SUVmax at the corresponding location of the biopsy on PSMA PET/CT was 30.28 (IQR: 18.80-48.49), with a SUVavg of 16.48 (IQR: 9.96-23.56).

Immunohistochemistry

All 22 patients showed tumor tissue on HE staining of their biopsy. The biopsy was taken from a bone metastasis in 14 (64%) patients, in 5 (23%) cases from a lymph node metastasis and in 3 (14%) cases from a visceral metastasis. Anywhere PSMA expression was present, the staining intensity was very strong (Figure 1). Therefore, no H-scores were calculated. Nineteen (86%) patients showed high and homogeneous (median 100%, IQR: 80%-100%) PSMA

expression on IHC of the entire tumor biopsy. One (5%) patient showed 40% PSMA expression of his tumor, while another patient showed only 1% PSMA expression on IHC. There was 1 (5%) patient without any PSMA expression although there was tumor on HE staining. The patient without PSMA expression showed a SUVmax of 21.58 and a SUVavg of 14.25 at the biopsy location. The patient with 1% PSMA expression showed a SUVmax of 18.73 and a mean SUVavg of 8.54 at the biopsy location. These patients both showed a lower SUV compared to the median SUVmax of the total group. The patient with a PSMA expression of 40% showed a SUVmax of 40.24 and a SUVavg of 19.79 at the biopsy location, this was higher compared to the median uptake of the total group. Overall, there was no correlation found between PSMA expression on IHC and the PSMA uptake on PSMA PET/CT at biopsy location (SUVmax: $R^2 = 0.046$ and SUVavg: $R^2 = 0.036$). Immunohistochemical Ki67 expression analysis was performed in 21 patients. The median Ki67 expression was 20% (IQR:

Table 2 Overview of the PSMA Uptake Results on PSMA PET/CT

Location	PSMA-Uptake on PSMA PET/CT
Biopsy	
SUVmax, median (IQR)	30.28 (18.80-48.49)
SUVavg, median (IQR)	16.48 (9.96-23.56)
Lymph node metastasis	
Mean SUVmax, median (IQR)	27.40 (18.01-35.90)
Mean SUVavg, median (IQR)	14.18 (9.28-19.47)
Bone metastasis	
Mean SUVmax, median (IQR)	43.76 (29.37-64.27)
Mean SUVavg, median (IQR)	22.97 (15.80-31.86)
Visceral metastasis	
Mean SUVmax, median (IQR)	14.48 (8.95-25.50)
Mean SUVavg, median (IQR)	6.97 (4.23-11.80)
Overall	
Mean SUVmax, median (IQR)	39.59 (23.17-47.07)
Mean SUVavg, median (IQR)	20.00 (11.96-25.85)

CT = computed tomography; IQR = interquartile range; PSMA = prostate specific membrane antigen; PET = Positron emission tomography; PSMA = prostate specific membrane antigen; SUVmax = standardized uptake value maximum; SUVavg = standardized uptake value average.

2%-50%) (Figure 3). Twelve (55%) patients showed a low Ki67 expression ($\leq 20\%$), whereas 9 (41%) patients had a high ($> 20\%$) Ki67 expression.

Outcomes After PSMA-RLT

Eighteen (82%) patients showed a PSA decline after PSMA-RLT, including 14 (64%) patients who had a PSA decline $> 50\%$ (Figure 4). Biochemical or clinical progression during follow up occurred in 21 (95%) patients, with a median PFS of 5.5 months (1-22). Twenty-one (95%) patients died during follow up. The median OS was 13.5 months (1-54). When comparing patients with low PSMA uptake versus patients with high PSMA uptake, no significant differences in PFS or OS were found (HR: 1.297, 95% CI: 0.490-3.439; $P = .604$), (HR 1.491, 95% CI: 0.553-4.019; $P = .430$). Of the 3 (14%) patients with low PSMA expression on IHC, the single patient without PSMA expression showed a PFS of 1 month and an OS of 3 months. The other 2 patients showed a PFS of 3 months and an OS of 6 and 7 months. Patients with high PSMA expression on IHC showed a significantly longer PFS (HR: 4.763, 95% CI: 1.135-19.990; $P = .033$). While a trend was also visible for OS, this was not statistically significant (HR: 3.654, 95% CI: 0.931-14.334; $P = .063$). Patients with a high Ki67 ($> 20\%$) expression showed a significant shorter OS compared to patients with low Ki67 ($< 20\%$) expression (HR: 0.278, 95% CI: 0.101-0.765; $P = .013$). A similar trend was also found for PFS (HR: 0.399, 95% CI: 0.151-1.055; $P = .064$). Survival curves are shown in Figure 5.

Discussion

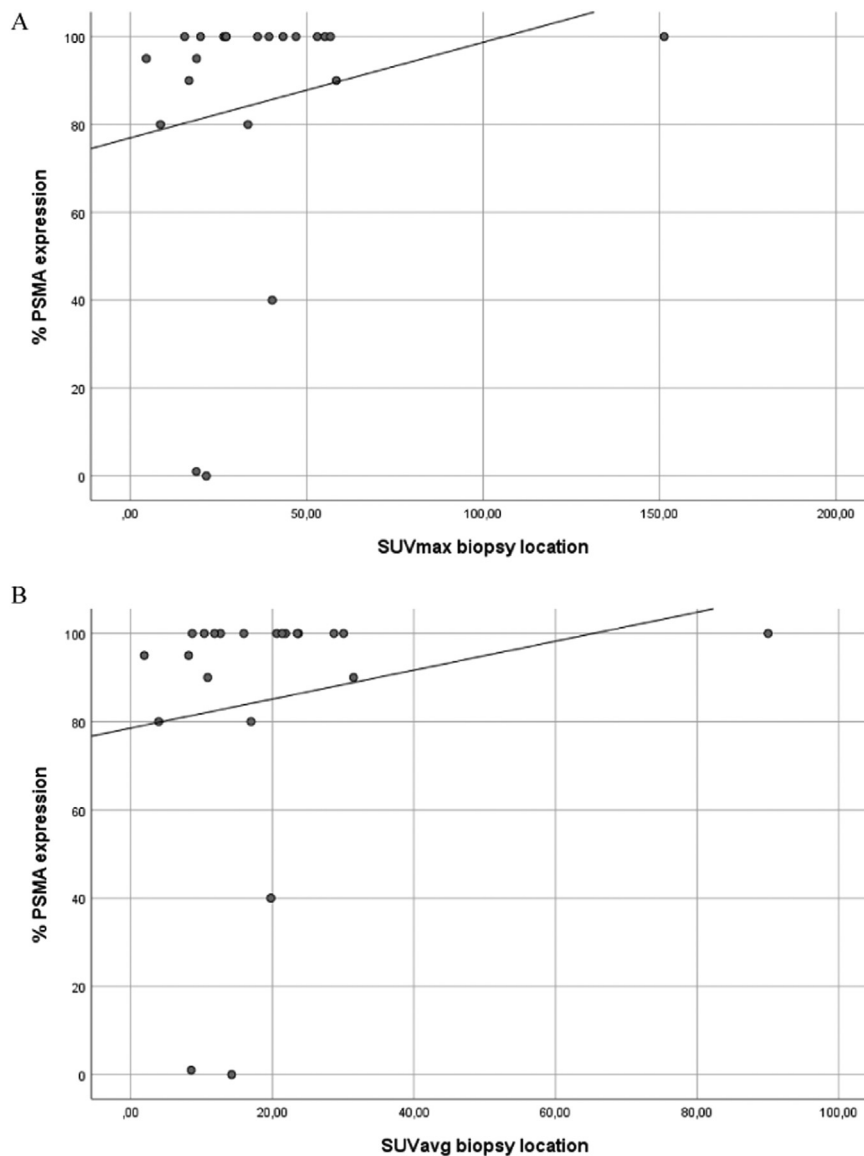
PSMA-RLT is a novel therapy for mCRPC patients with tumors that express high levels of PSMA on PSMA PET/CT. Unfortunately, there is a varying response between patients with only about 60% of patients having clinical benefit.^{4,5,30} To date, several predictive

factors for PSMA-RLT response have been studied, but the differing response remains poorly understood.¹³⁻²³ The present study aimed to improve the understanding of the varying therapeutic response by evaluating fresh biopsies prior to PSMA-RLT therapy. This study observed that the PSMA ligand uptake on PSMA PET/CT acquired prior to PSMA-RLT may not reveal PSMA expression heterogeneity on IHC. This intratumoral heterogeneity may affect treatment outcome. Moreover, this study observed that a high Ki67 expression is a negative predictor of the therapeutic outcome following PSMA-RLT.

Only those patients with high (tumor to liver ratio > 1.0 and SUVmax > 15) and macroscopically homogenous PSMA uptake on PSMA PET/CT were considered eligible for PSMA-RLT. Thus, all included patients had a high tumor PSMA uptake on PSMA PET/CT. While PSMA uptake on PSMA PET/CT imaging generally followed the PSMA expression on IHC, 3 of the 22 patients had a poor homogeneity of PSMA expression in their tumor biopsy even though tumor was present on histology and significant PSMA avidity on PET/CT was seen (Figure 3). This is in line with a recent preclinical study that observed a heterogeneity in PSMA expression on IHC in 84% of prostate cancer tissue samples.³¹ These 3 patients also showed a worse response to PSMA-RLT compared to the other 19 patients with better PSMA expression (2.3 vs. 8.1 months, and 5.3 vs. 20.4 months, respectively). These patients may have had a microscopic heterogeneous tumor with an important (poorly differentiated) cell line without PSMA expression which became dominant following PSMA-RLT.

Overall, patients with highly proliferating tumors have poor prognosis due to the rapid reproduction of cancer cells.²⁷ Although actively dividing cells may be more vulnerable for radiation therapies such as PSMA-RLT, we observed a significantly longer OS, and a tendency to longer PFS for patients with low ($< 20\%$) Ki67 expression. This is in line with previous reports on ¹⁷⁷Lu-Octreotide

Figure 3 : PSMA expression is not correlated to the PSMA uptake on the biopsy location for both (A) SUVmax: $R^2 = 0.046$ and (B) SUVavg: $R^2 = 0.036$. The 3 patients with low PSMA expression (0/1/40% expression) show an evident PSMA uptake at biopsy location. PSMA = prostate specific membrane antigen; SUVmax = standardized uptake value maximum; SUVavg = standardized uptake value average.

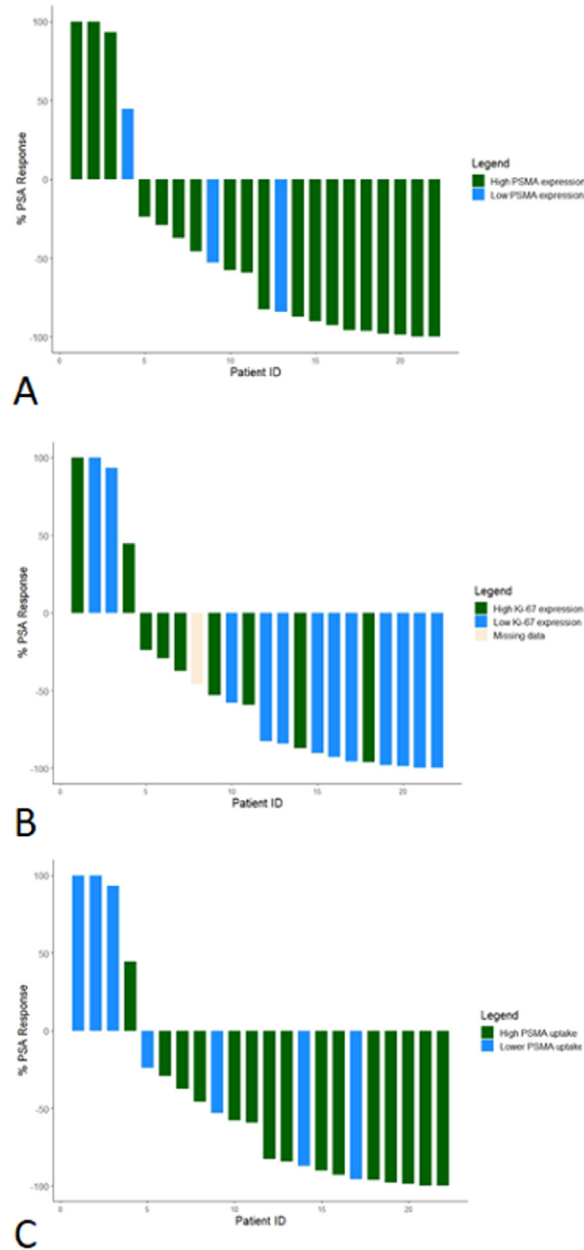


RLT in neuroendocrine tumors.^{32,33} Although its relationship is more complex, increased cellular proliferation is linked to a higher FDG uptake. It is known that a higher FDG uptake is also a negative predictor of PSMA-RLT outcome.³⁴⁻³⁶ All in all, a larger prospective trial is needed to confirm if Ki67 can be used as a prognostic marker to exclude patients from ineffective PSMA-RLT.

A single site biopsy may be not fully representative for all metastases due to intratumoral and intrapatient heterogeneity. However, it may provide additional understanding on a cellular level, partic-

ularly when a fresh tumor sample is available such as in primary prostate cancer patients (PSMAddition study NCT04720157 and LuTectomy NCT04430192). Unfortunately, evaluation on a cellular level is not possible with molecular imaging such as SPECT or PET/CT imaging due to the resolution and spill-over effect. Therefore, assessing the tumor sample may provide important prognostic data. In other tumor types, IHC is also used to provide predictive information for treatment with immune checkpoint inhibitors targeting PD (L)-1 and epidermal growth factor receptor inhibitors.^{37,38}

Figure 4 : Waterfall plots showing PSA responses. A: High versus low PSMA expression, B: high versus low Ki-67 expression, C: high versus low PSMA uptake. PSMA = prostate specific membrane antigen.

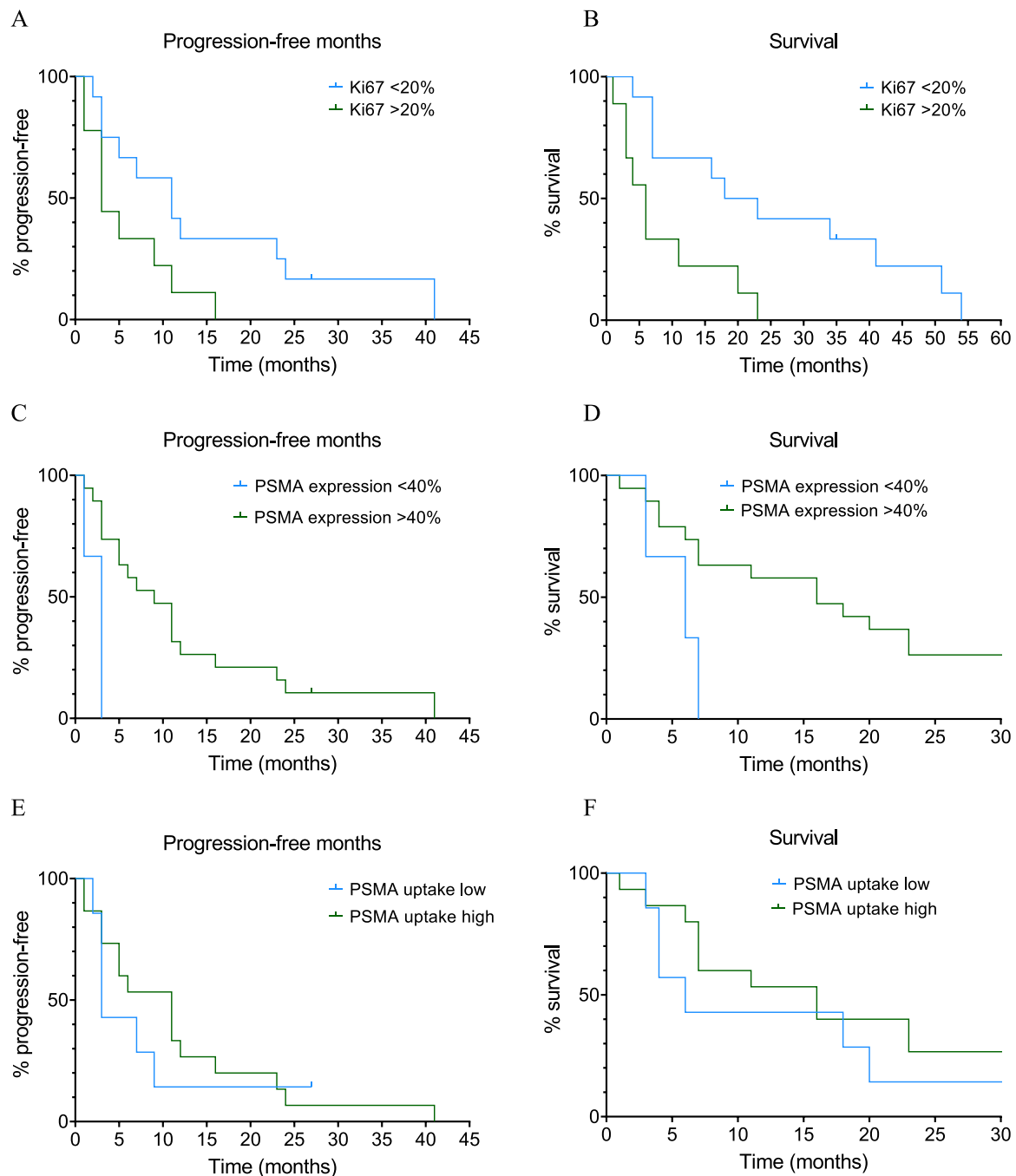


We cannot draw strong conclusions from this explorative study. Therefore, the present findings need to be further investigated in a larger prospective cohort study. The study is limited by its small, heterogeneous cohort and its retrospective study design. Moreover, the application of both [^{68}Ga]Ga-PSMA-11 and [^{18}F]PSMA-1007 PET/CT may have introduced bias in the SUV measurements.

Nevertheless, the presented outcomes provide suggestions for future studies.

In conclusion, the PSMA uptake on PSMA PET/CT and the PSMA expression on IHC is high in mCRPC patients undergoing PSMA-RLT. Immunohistochemical PSMA and Ki67 expression in fresh tumor biopsies may contribute to predict treatment efficacy of

Figure 5 : Survival curves (A-F); Progression free months and overall survival. PSMA = prostate specific membrane antigen.



PSMA-RLT in mCRPC patients. However, this needs further evaluation in prospective studies.

Clinical Practice Points

- PSMA directed RLT is a novel therapy for mCRPC patients. However, it is still poorly understood why approximately 40% of the patients do not respond to PSMA-RLT, while others have

an excellent response, despite all having high uptake on PSMA-PET/CT prior to treatment.

- We found that the PSMA uptake on PSMA PET/CT is high in mCRPC patients undergoing PSMA-RLT and generally follows the PSMA expression on IHC. However, patients with a low PSMA expression had a shorter PFS compared to patients with high PSMA expression on IHC. Patients with low Ki67

expression had a longer PFS and OS compared to patients with a high Ki67 expression. Therefore, IHC data from fresh tumor biopsies may contribute to predict treatment efficacy of PSMA-RLT in mCRPC patients.

Disclosure

The authors have declared that no competing interest exists.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clgc.2023.04.003.

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